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First records of marine tardigrades of the genus *Coronarctus* (Tardigrada, Heterotardigrada, Arthrotardigrada) from Mexico

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Abstract

Deep-water sampling in the Perdido Fold Belt, Gulf of Mexico, Mexican Economic Exclusive Zone yielded five specimens of tardigrades belonging to the genus *Coronarctus* Renaud-Mornant, 1974. The specimens represent the first records of the genus for Mexico. Two two-clawed larvae and two four-clawed larvae of *Coronarctus mexicus* Romano, Gallo, D'Addabbo, Accogli, Baguley & Montagna, 2011 and a single four-clawed larval specimen of an undescribed *Coronarctus* species were identified. Taxonomic analysis of the specimens contributed to the knowledge of deep-sea and Mexican marine tardigrades, two data-poor areas of study.

Keywords

Coronarctidae; biodiversity; meiofauna.

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Introduction

The Gulf of Mexico, an ocean basin boarded by the coasts of the United States of America, Cuba, and Mexico, is among the world's most species rich marine regions as evaluated by the Census of Marine Life (2000–2010) (Costello et al. 2010). Yet, the actual extent of global marine species diversity, particularly within deep-sea habitats, remains under-sampled and undocumented (Hendriks et al. 2006; Web et al. 2010; Mora et al. 2011). This is partly due to a predominating focus of global biodiversity research efforts on terrestrial systems (Hendriks and Duarte 2008). Consequently, many marine organisms are poorly known, including those that comprise the meiofauna (Snelgrove 1999).

Tardigrades (Phylum Tardigrada) are represented within meiofaunal communities of both marine and freshwater sediments. Information on all aspects of marine tardigrade biology, including species diversity and global geographic distribution patterns, is considered particularly deficient (Fontoura et al. 2017) with many regions remaining wholly unstudied (Kaczmarek et al. 2015). The amount of available data concerning marine tardigrades in the Gulf of Mexico, especially in its noncoastal zones, is limited.

While several deep-sea studies in this region have included Tardigrada in phylum level compositional analyses of meiofaunal communities (e.g. Baguley et al. 2006; Landers et al. 2012; Reuscher et al. 2017; Cisterna-Céliz et al. 2019), these did not include identifications at lower taxonomic levels. Thus far, only 15 tardigrade taxa have been recorded from the Gulf of Mexico (Romano 2009 and references therein; Romano et al. 2011; Santos et al. 2019). Six taxa are known from coastal studies in Alabama and Florida, USA (Chitwood 1951, 1954; King 1962; Riggin 1962; McKirdy 1975; Romano 2009; Santos et al. 2019). A single offshore investigation focusing on the Gulf's northern continental slope and abyssal plain recovered the remaining nine taxa (Romano et al. 2007a, 2007b; Romano et al. 2011). All of these records originate from localities within the USA territorial sea boundary or Economic Exclusive Zone (EEZ).

The first records for Mexican marine tardigrade taxa were obtained only recently by Pérez-Pech et al. (2018) who reported the genera *Archechiniscus* Schulz, 1953, *Batillipes* Richters, 1909, *Dipodarctus* Pollock, 1995, *Echiniscoides* Plate, 1888, and *Wingstrandarctus* Kristensen, 1984 from Mexican Caribbean coastal sites. The Gulf of Mexico and Pacific waters within Mexico's maritime boundaries had thus far completely lacked distribution records for marine tardigrade taxa.

In 2016 and 2017, the oceanographic vessel *Justo Sierra* explored the deep-water environment of the Perdido Fold Belt, Gulf of Mexico, a petroleum reservoir-containing province, with the objective of evaluating the potential use of meiofauna as a bio-indicator for the impact of future oil exploration and extraction in the area. Tardigrade specimens belonging to the genus *Coronarctus* Renaud-Mornant, 1974 were recovered from the collected sediments. These specimens represent the first species records of marine tardigrades from the Gulf of Mexico, Mexican Economic Exclusive Zone.

Methods

Sediment collection was carried out on board *Justo Sierra* in the Mexican zone of the Perdido Fold Belt. Sediments were collected at 21 stations ranging in depth from 116–3,447 m (Fig. 1) with a Hessler grab (40 × 40 × 60 cm). A single sample from the processed sediment from each station was taken using a corer (10 cm internal

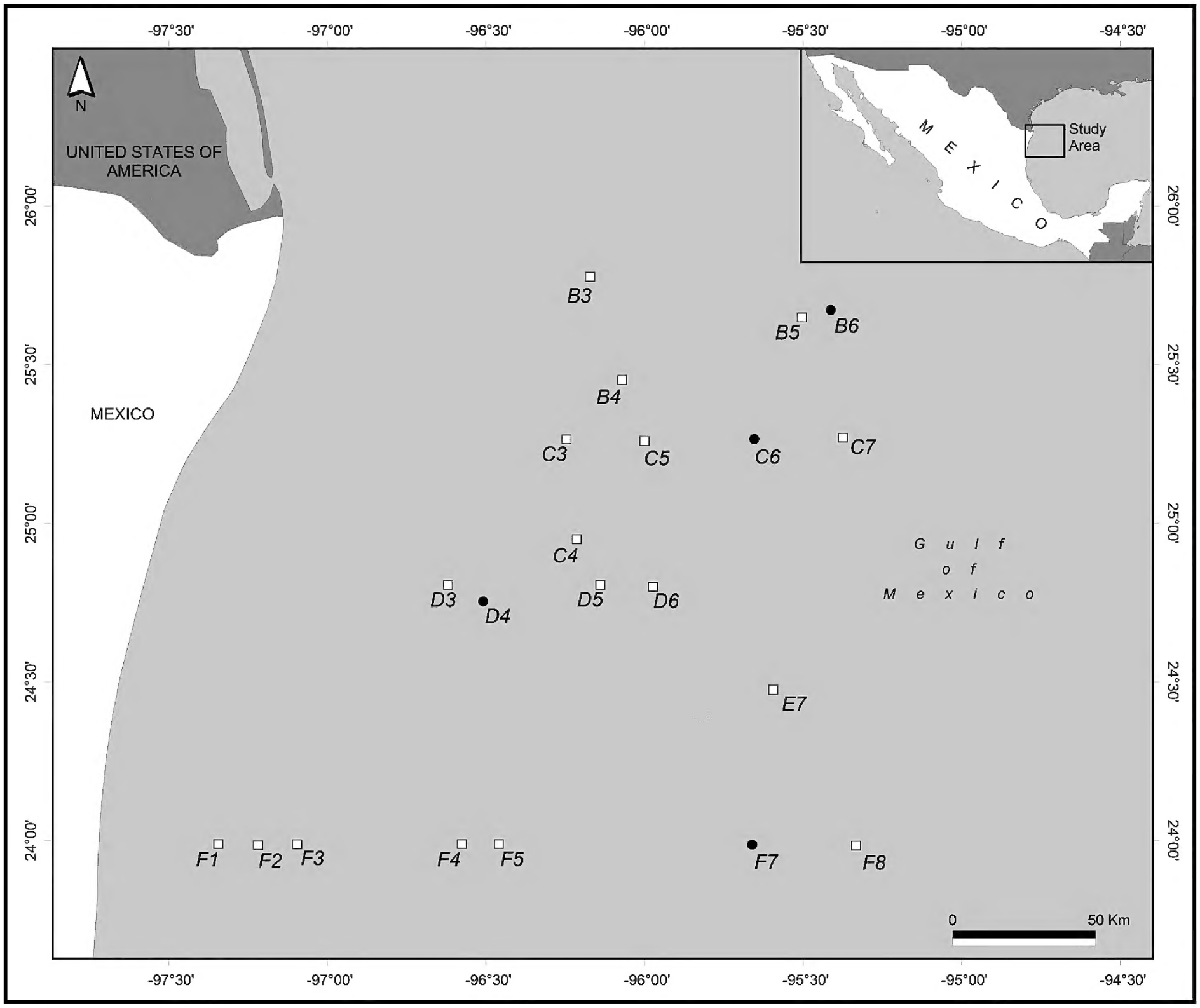

Figure 1. Sampling stations, Perdido Fold Belt, northwest Gulf of Mexico. Filled dots indicate stations positive for tardigrades.

diameter, 10 cm length) and fixed immediately with 10% neutralized formalin. In the laboratory, the samples were washed with filtered tap water and processed by flotation method (sensu Nichols 1979) using a LUDOX solution (density of 1.25). Meiofauna were collected on a 45 µm sieve, then manually sorted using a stereomicroscope. Tardigrade specimens were mounted on individual glass slides beneath a coverslip. Prior to sealing slides with nail polish, mounted specimens were treated through a series of glycerin concentrations (10, 25, and 75%) to complete the preservation process.

Observations for taxonomic identification were made with an Axio Lab A1 Carl Zeiss or Olympus BX51 microscope with magnification up to 100× using differential interference contrast (DIC) or phase contrast (PhC). Microphotographs and measurements were taken with CellD (dp20) software. Identifications were made by comparisons with type material and the relevant primary literature (see below). Type material observed in this study for comparative purposes is housed at Muséum National d'Histoire Naturelle, Paris (MNHN) (*Coronarctus tenellus* Renaud-Mornant, 1974; *C. stylisetus* Renaud-Mornant, 1987; *C. fastigus* Renaud-Mornant, 1987; *C. laubieri* Renaud-Mornant, 1987; *C. disparilis* Renaud-Mornant, 1987) and the Natural History Museum of Denmark (NHMD) (*C. verrucatus* Hansen, 2007). Material representing new records reported in the present study was deposited in the collections at NHMD and El Colegio de la Frontera Sur-Unidad (ECOSUR), Chetumal, Mexico.

Results

The collected sediment from each station was composed of fine silt with the average grain size diameter ranging from 44.8–69.5 µm and the percentage of organic material ranging from 6.3–14%. Five tardigrade specimens were extracted from 4/21 samples from stations: B6 (2 specimens), C6 (1 specimen), D4 (1 specimen) and F7 (1 specimen). The extracted tardigrades all belong to the genus *Coronarctus*, represented by *Coronarctus mexicus* and an undescribed *Coronarctus* sp.

Coronarctus mexicus Romano, Gallo, D'Addabbo, Accogli, Baguley & Montagna, 2011

New records. Mexico • Gulf of Mexico: Station B6 (25°37.97′N, 095°25.86′W; 1847 m depth), Sara Berenice Balán Zetina and Anabel León Hernández leg., 22 Sep. 2017 (1 two-clawed larva, ECOSUR-2208201701) • Gulf of Mexico: Station C6 (25°15.85′N, 095°39.28′W; 1810 m depth), Sara Berenice Balán Zetina and Anabel León Hernández leg., 22 Sep. 2017 (1 two-clawed larva, NHMD-618112) • Gulf of Mexico: Station D4 (24°47.99′N, 096°30.05′W; 800 m depth), Sara Berenice Balán Zetina and Anabel León Hernández leg., 20 May 2016 (1 four-clawed larva, ECOSUR-2005201601) • Gulf of Mexico: Station F7 (23°59.13′N, 095°39.67′W; 2847 m depth), Sara Berenice Balán Zetina and Anabel León Hernández leg., 27 Sep. 2017 (1 four-clawed larva, NHMD-618114).

Identification. Station B6: 1 two-clawed larva, length 291 µm, width 82 µm; Station C6: 1 two-clawed larva, length 232 µm, width 64 µm; Station D4: 1 four-clawed larva, length 330 µm, width 83 µm; Station F7: 1 four-clawed larva, length 376 µm, width 98 µm.

The collected specimens conform to the descriptions given by Romano et al. (2011) in all observed characters of either the first-stage larvae (two-clawed) or the second-stage larvae (four-clawed) (Fig. 2) in terms of both qualitative characters and morphometric values for measurable structures. The claw measurements of both two-clawed larva specimens were (external, internal): leg I (21 µm, 24 µm); leg II (16 µm, 19 µm); leg III (14 µm, 17 µm); Leg IV (35 µm). Due to unsuitable orientations, the claw measurements of only one of the four-clawed larvae were possible. These were: leg I (23 µm, 29 µm); leg II (17 µm, 24 µm); leg III (15 µm, 24 µm); leg IV (47 µm).

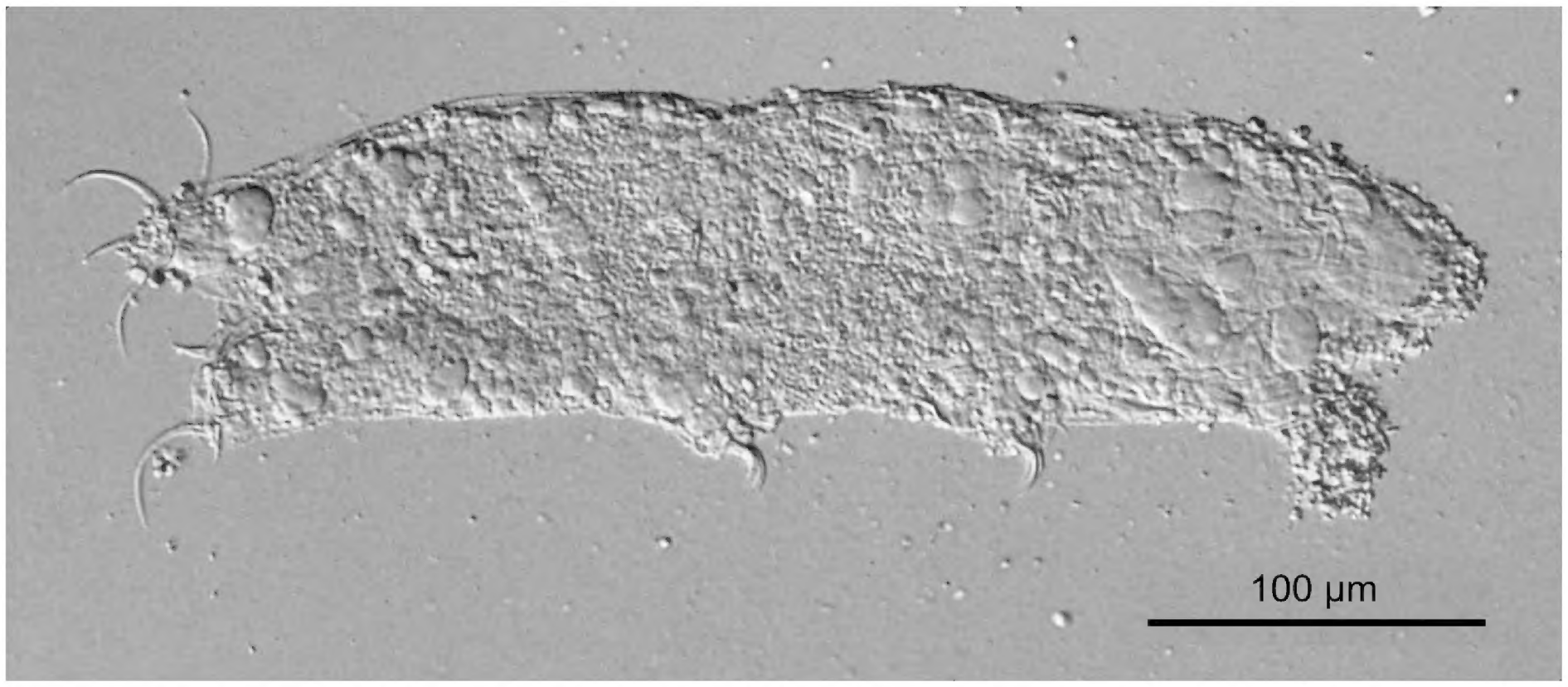

Figure 2. *Coronarctus mexicus*, four-clawed larva. DIC.

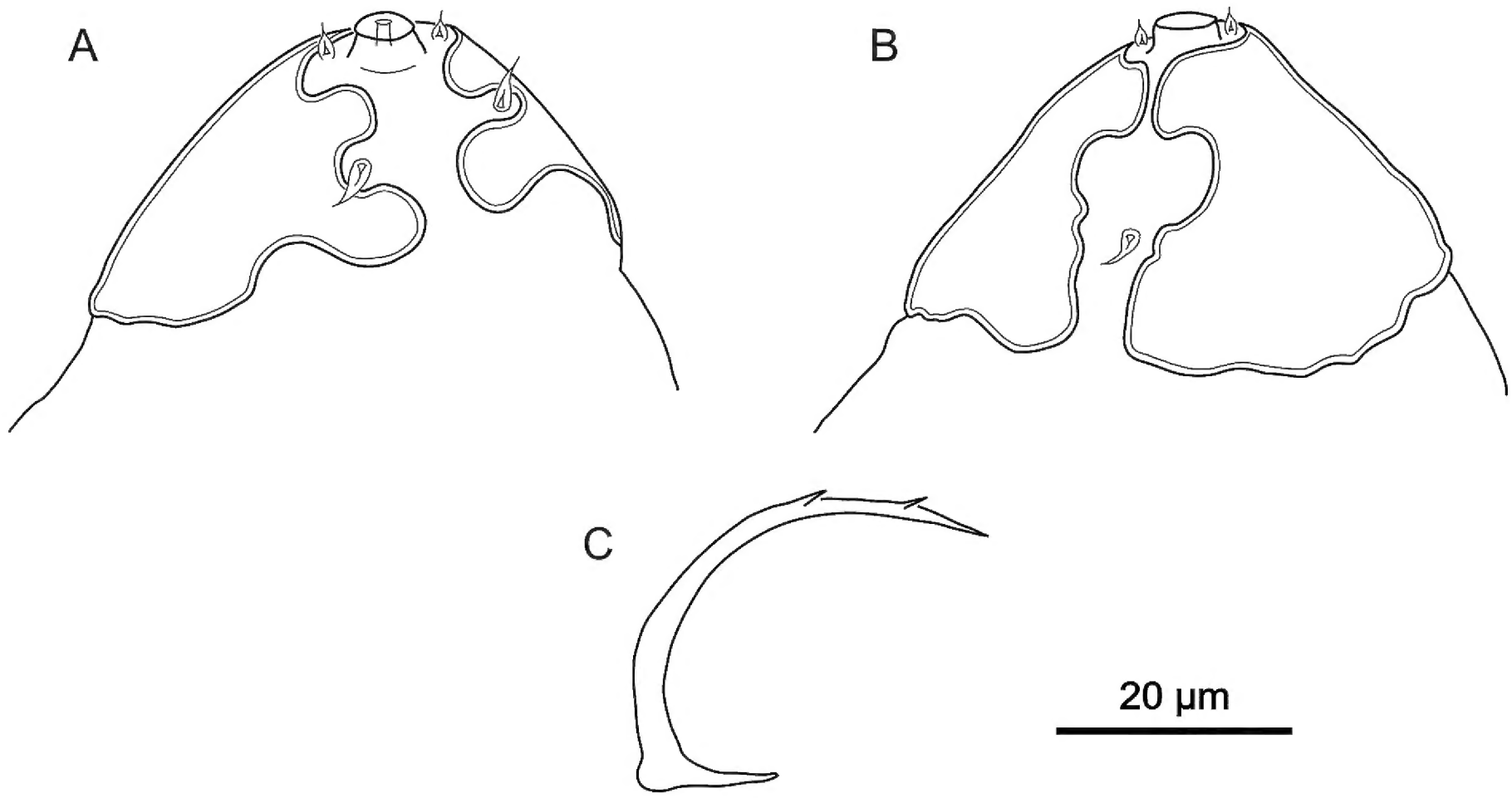

Figure 3. *Coronarctus mexicus*, four-clawed larva. **A.** Secondary clavae, ventral view. **B.** Secondary clavae, dorsal view. **C.** Claw, leg IV.

The smallest specimen lacks both anus and gonopore, while the anus, but not the gonopore, were recognized in the other three specimens. The large size difference between each specimen indicates at least four molts during larval development of this species.

In the description of *Coronarctus mexicus*, Romano et al. (2011) included only a limited illustration of the secondary clavae in ventral view. As the shape of these structures is species- specific and therefore crucial in discriminating between *Coronarctus* species, new detailed illustrations of the secondary clavae of the four-clawed larva in both ventral (Fig. 3A) and dorsal view (Fig. 3B) are provided. Furthermore, the presence of an additional dorsal accessory spine on the claws of leg pair IV (Fig. 3C) was recognized on the new specimens. Romano et al. (2011) described only a single accessory spine on each claw. The second accessory spine was likely overlooked by the describing authors, as the visibility of this minute structure depends strongly on the orientation of the claw.

Undescribed *Coronarctus* sp.

New record. Mexico • Gulf of Mexico: Station B6 (25°37.97′N, 095°25.86′W; 1847 m depth), Sara Berenice Balán Zetina and Anabel León Hernández leg., 22 Sep. 2017 (1 four-clawed larva, NHMD-618113).

Identification. Undescribed *Coronarctus* sp.. Station B6: 1 four-clawed larva, length 398 µm, width 161 µm.

Following a comparison to the holotypes of *C. tenellus*, *C. stylisetus*, *C. fastigus*, *C. laubieri*, *C. disparilis*, *C. verrucatus*, and the original description of *C. mexicus*, the specimen found in the present study (Fig. 4) could not be assigned to any known taxon due to the unique form of the contours of the secondary clavae (Fig. 5A, B). It clearly belongs to the group of *Coronarctus* species displaying claw heterometry (*C. tenellus*, *C. laubieri*, *C. disparilis*, and *C. mexicus*), as the internal claws are slightly longer than external claws on leg pairs I–III and twice as long as external claws on leg pair IV. Other notable characters of this undescribed species are the remarkable body proportions. With a body length of 398 µm it is a fairly large *Coronarctus* species. Only adults of *C. laubieri* have been reported to exceed a body length of 400 µm (Renaud-Mornant 1987, 1988). The maximum body width of any *Coronarctus* species previously reported was 110 µm (i.e. *C. laubieri* (Renaud-Mornant, 1987)). The observed characters clearly separate this specimen from all known *Coronarctus* species. However, a formal description cannot be completed until corresponding adult specimens are obtained.

Discussion

The recovery of the specimens examined in the present study adds two new taxa, *Coronarctus mexicus* and an undescribed *Coronarctus* sp., to the known Mexican tardigrade fauna. These specimens are the first Mexican representatives of the genus to be reported. Four of nine taxa recorded by the only other noncoastal survey to report tardigrade species from the Gulf of Mexico (Romano et al. 2011) were also *Coronarctus* species. These were *C. disparilis*, *C. laubieri*, *C. stylisetus*, and *C. mexicus*. Romano et al. (2011) recorded *C. mexicus* adults, two-clawed and four-clawed larvae from several of their sampling stations, although the numbers of specimens represented by each life stage and the locations of the sampling stations from which they were collected were not specified. In the present study all collected tardigrade specimens were juveniles. The recovery of larvae of multiple stages from the deep-sea localities of the present study and of Romano et al. (2011) supports the ideas of Renaud-Mornant (1988) regarding

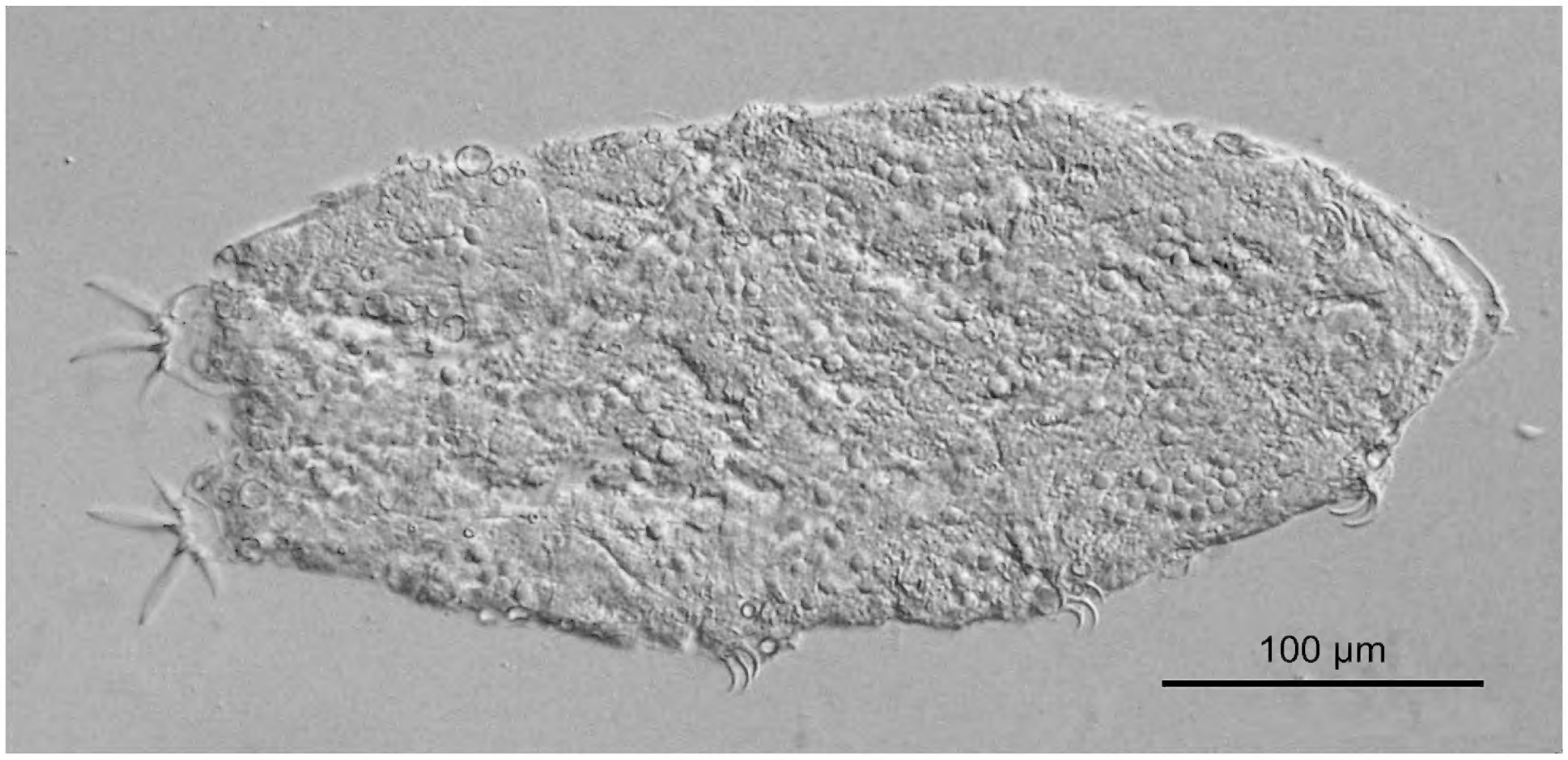

Figure 4. Undescribed *Coronarctus* sp., four-clawed larva. DIC.

post-embryonic development of deep-sea dwelling tardigrades. Specifically, Renaud-Mornant (1988) postulated that in comparison to that typical of tardigrades in other marine habitats, ontogeny in the deep-sea involves both a greater number of molts in each larval stage and growth to a larger body size before each molt due to the local scarcity of available food. The remarkable body proportions of the undescribed *Coronarctus* sp. larva reported herein are particularly demonstrative of the latter component of Renaud-Mornant's hypothesis.

In the only two studies to include species level identifications of deep-sea tardigrades in the Gulf of Mexico, Romano et al. (2011) and the present study, previously unknown taxa were encountered. This could indicate a potential for further taxonomic novelties to be recovered with increased sampling in the Gulf of Mexico. The descriptions provided above of the juvenile *Coronarctus* sp. specimen might be useful in ascribing that larva to an adult form collected in future sampling campaigns, following which the species may be formally described.

While only five tardigrade specimens were collected in the present survey, this is not considered to be unusual. Generally, tardigrades are not a dominant component of the meiofauna and the abundance of marine tardigrade taxa is typically low within samples (Jørgensen et al. 2010; Fontoura et al. 2017). In the Gulf of Mexico deep-sea, Tardigrada is also consistently reported as a minor contributor to meiofaunal community composition (Baguley et al. 2006; Landers et al. 2012; Reuscher et al. 2017; Cisterna-Céliz et al. 2019). Additionally, Romano et al. (2011) reported a low average abundance of tardigrades per positive sample (1.5 specimens/100 cm^3 sample).

In the present study, the distribution of tardigrades between collections stations may be patchy, as few samples contained tardigrades and no station that was positive for tardigrades was adjacent to any other positive station. This patchiness of distribution is commonly reported for tardigrades, both in the marine (e.g. Hansen et al. 2001; Bartels et al. 2018) and terrestrial environments (e.g. Meyer 2006; Degma et al. 2011) and indicates a need for a sufficient quantity of samples when determining the presence or absence of tardigrades in a particular area.

The genus *Coronarctus*, although infrequently recorded, has demonstrated a broad geographic distribution. In addition to the present records and those of

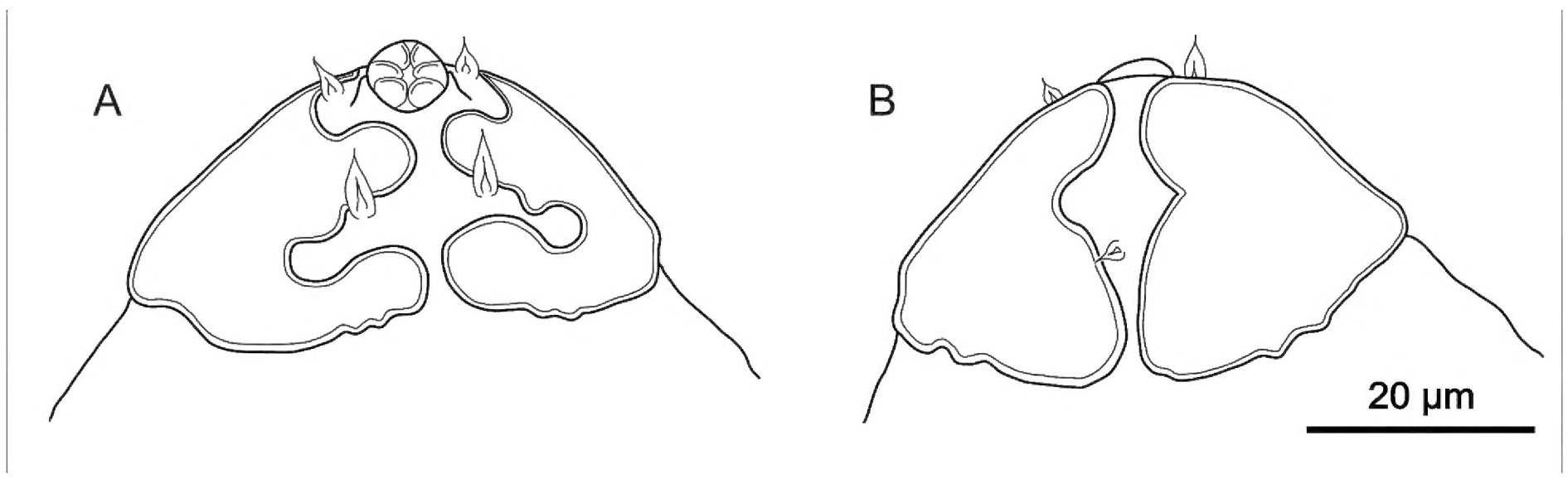

Figure 5. Undescribed *Coronarctus* sp., four-clawed larva. **A.** Secondary clavae, ventral view. **B.** Secondary clavae, dorsal view.

Romano et al. (2011) from the Gulf of Mexico, *Coronarctus* species have been reported from the Indian Ocean (Renaud-Mornant 1974, 1975, 1987), Asian Pacific Ocean (Noda 1989; Fujimoto et al. 2017), North American Atlantic (Renaud-Mornant 1987), European Atlantic (Renaud-Mornant 1987; Hansen 2007), African Atlantic (Renaud-Mornant 1974, 1975), and the Black Sea (Kharkevych 2013).

The *Coronarctus* species collected in the present study were retrieved from stations ranging in depth from 800–2847 m, a range overlapping with the 625–3150 m depth range given for the *Coronarctus* species collected by Romano et al. (2011). All Gulf of Mexico records of *Coronarctus*, including the present new records of *C. mexicus* and the undescribed *Coronarctus* sp. larvae from the Mexican EEZ, support Kaczmarek et al. (2015) in their regard of *Coronarctus* as a deep-sea genus. However, the dataset of those authors did not include shallow depth records for *Coronarctus* spp. from <100 m in the Black Sea (Kharkevych 2013), 29 m in a Japanese submarine cave (Fujimoto et al. 2017), and 40 m depth in Tanabe Bay, Japan (Noda 1989). Additionally, one of the seven known *Coronarctus* species, *C. verrucatus*, was collected at depths between 249–260 m from Faroe Islands, European Atlantic (Hansen 2007). With increased sampling the zonal distribution of *Coronarctus* could prove to be more similar to that of *Angursa* Pollock, 1979, which has been recorded from the shallow subtidal to abyssal zones (Fujimoto and Hansen 2019).

Further sampling campaigns paired with in-depth taxonomic studies are greatly needed in order to catalogue and quantify biodiversity in Mexican marine ecosystems (Salazar-Vallejo et al. 2007; Bastida-Zavala et al. 2013). Data on tardigrades from Mexico's Pacific maritime territory are still nonexistent. Given the current scarcity of distributional data for marine tardigrades both in Mexican waters and globally, at present it is not possible to make firm conclusions about species ecologies. For this reason, any future records will have importance in contributing to the understanding of marine tardigrade diversity and distribution.

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Authors' Contributions

WAPP and JGH compiled information on characters, identified specimens, prepared figures, interpreted data, and contributed to manuscript drafts. JGH provided all illustrations. ED conducted background research, prepared figures, and produced the final manuscript draft. AdJN participated in fieldwork, prepared figures, interpreted data and contributed to manuscript drafts. MMI, ROA, and VEA participated in fieldwork and revisions of manuscript drafts.

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